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Impact of Deliquescence on the Chemical Stability of Vitamins B_1, B_6, and C in Powder Blends

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Single vitamin ingredients and blends in premixes are widely used in the food and supplement industries and are predominantly in powder form. To meet label claims and/or determine appropriate overages, it is important to characterize the stability of these ingredients. Although moisture is a known promoter of instability in powder blends, the combined effects of storage relative humidity (RH), formulation, and deliquescence on the stability of these systems are not well-characterized. The objective of this study was to determine the effect of deliquescence on the stability of vitamins B_1, B_6, and C and their mixtures. Deliquescence points (RH_0s) for all formulations were determined by moisture sorption analysis. Single, binary, ternary, and quaternary mixtures of thiamin HCl, pyridoxine HCl, sodium ascorbate, and fructose were stored in RH-controlled environmental chambers between 43 and 98% RH at 22 °C for up to 12 weeks. Vitamin stability was determined by high-performance liquid chromatography (HPLC). Formulation and storage RH significantly affected vitamin stability. Thiamin and ascorbate degradation were significantly promoted above the RH_0, while pyridoxine was least affected by storage RH. The deliquescence lowering phenomenon enhanced moisture sorption of blends at RHs below the RH_0s. Ascorbate enhanced thiamin degradation. Therefore, formulation, storage conditions, and the relation of these to deliquescence points may affect the shelf life, quality, and functionality of vitamin blends and should be considered in product development, processing, storage, and use.

KEYWORDS: Deliquescence; thiamin; pyridoxine; ascorbate; stability; humidity

INTRODUCTION

The predominantly powder or granular dietary supplement formulations and premixes that contain bioactive ingredients comprise large segments of the U.S. and global economy. U.S. dietary supplement sales totaled an estimated 4.7 billion in 2006 (*1*). Vitamins are the largest supplement category, capturing 18% of the supplement market (*2*), and multivitamins are the most commonly consumed supplement type (*1*). Vitamin C is the third most commonly consumed single vitamin, consumed by 9.4% of adults, and B-complex vitamins are used by 4.3% of consumers. Vitamins C, B_1, and B_6 are also frequently incorporated into foods and premixes, and their concentrations are declared on food labels. These water-soluble vitamins are highly susceptible to degradation under common storage and processing conditions. Ascorbic acid and thiamin are among the least stable vitamins. Their degradation is influenced by pH, oxygen, water activity, and the food matrix (*3*). Because the addition of vitamins to foods and supplements is intended to deliver these nutrients to the consumer, and vitamins are subject to food labeling laws, controlling vitamin stability/degradation and uniformity in foods and supplements is important.

Water plays an important role in influencing degradation rates of water-soluble vitamins, and an increase in moisture content contributes to decreased vitamin stability (*4*–*7*). While these interactions have been studied in liquid systems, the contribution of specific water−solid interactions that occur in powder blends containing vitamins, such as deliquescence, to chemical and physical instabilities in powder blends containing vitamins has not been reported. Deliquescence is a first-order phase transformation from solid to solution that occurs at a certain relative humidity (RH) that is specific to that crystalline solid. The deliquescence RH is termed RH_0. The phenomenon of deliquescence in multicomponent organic systems is complex and has not been widely investigated. Recently, the phenomenon of deliquescence has been applied to food-related studies (*8*). Thiamin HCl and sodium ascorbate have been established as deliquescent ingredients (*8, 9*). In addition, a phenomenon known as deliquescence lowering has been witnessed with mixtures of sugars and vitamins, as well as sugars and inorganic

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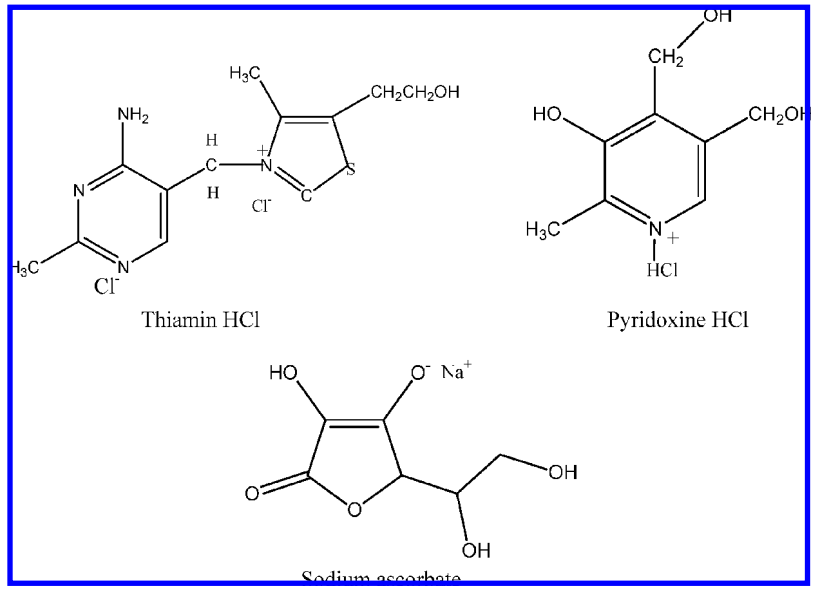

Figure 1. Structures of thiamin HCl, pyridoxine HCl, and sodium ascorbate.

salts (*8*). Deliquescence lowering is a reduction in the RH where the solid to solution transition occurs, termed RH_{0mix}, when deliquescent compounds are mixed. This presents concern for products that contain a mixture of deliquescent ingredients because the RH_{0mix} is lower than any of the individual ingredient RH_0s. Deliquescence lowering has been shown to enhance chemical reactivity at RHs commonly encountered in food processing facilities (*10*). Moisture introduced into the system increases reactant mobility and chemical instability. Therefore, deliquescence may affect the shelf life, quality, and functionality of food products that contain deliquescent ingredients.

Understanding the physical and chemical properties of multicomponent food powders that contain vitamins is necessary to supply consumers with high quality, value-added products. Premixes and dietary supplements often contain mixtures of deliquescent ingredients (vitamins, organic acids, sugars, salts), and it is important to understand how these ingredient blends will behave throughout formulation, processing, packaging, storage, distribution, and the resulting quality and shelf life of the product. The hydrochloride forms of both thiamin and pyridoxine are commonly used in food supplementation (*3, 11*). Sodium ascorbate is often used as a source of vitamin C in foods and dietary supplements. Thus, an understanding of the impact of water−solid interactions on chemical and physical stability within food powders is important for ensuring delivery of the vitamins in their primary active forms. The objective of this work was to determine the effect of deliquescence and deliquescence lowering, storage RH conditions, and formulation on the chemical stability of the water-soluble vitamins sodium ascorbate, thiamin HCl, and pyridoxine HCl (**Figure 1**).

MATERIALS AND METHODS

Materials. Ingredient forms of water-soluble vitamins were selected based on common usage and varying interactions with water. Thiamin hydrochloride was purchased from Mallinckrodt-Baker (Philipsburg, NJ). Sodium ascorbate, pyridoxine hydrochloride, and fructose were obtained from Sigma Aldrich Co. (St. Louis, MO). Fructose was chosen as a representative deliquescent, nonvitamin ingredient. The following salts were used to create saturated salt solutions and control RH in environmental chambers: K_2CO_3, $CoCl_2$, KCl, and K_2SO_4 (Mallinckrodt-Baker, Phillipsburg, NJ); NaBr (Fisher Scientific, Fair Lawn, NJ); $Mg(NO_3)_2$ (Sigma-Aldrich Inc., St. Louis, MO). Certified HPLC and ACS grade methanol (Mallinckrodt-Baker, Phillipsburg, NJ) and trifluoroacetic acid (TFA) (Sigma-Aldrich Inc., St. Louis, MO) were used.

Water Activity Measurements. Samples were prepared by adding 200−500 μL of distilled water to 1−2 g of powder (binary, ternary, and quaternary mixtures were prepared by geometric mixing of equal mass ratios of multiple ingredients). Water activity (a_w) of samples was measured in a chilled mirror dewpoint instrument (AquaLab 3TE, Decagon, Pullman, WA) at 25 °C after a 48 h sample equilibration time. The a_w of the saturated solution was used as a prediction of the deliquescence RH (RH_0 or RH_{0mix}) (*9*).

Moisture Sorption Isotherms. Gravimetric sorption analysis was performed using a symmetrical gravimetric analyzer (SGA-100) (VTI Corporation, Hialeah, FL) at 25 °C in order to determine the critical relative humidity of deliquescent solids (RH_0) and mixtures (RH_{0mix}) (*9*). For the mixtures, larger quantities of individual components were blended at an equal mass ratio, and a 15−20 mg portion of the sample was used for analysis. Prior to sorption analysis, samples were dried at 60 °C in the sorption analyzer. The settings for the sorption analyzer were equilibrium criterion for the drying step of 0.01% w/w in 2 min, maximum drying time of 30 min, and step equilibrium criterion of 0.001% w/w in 5 min with a maximum step time of 30 min. During the experiment, samples were exposed to increasing RH (from 0 to 94% RH), increasing at 2% intervals from 40 to 94% RH. RH_0 and RH_{0mix} were determined from the point in the isotherm at which the sample began to rapidly sorb moisture (*9*).

Controlled Relative Humidity Storage. Single, binary, ternary, and quaternary mixtures of sodium ascorbate (A), fructose (F), thiamin HCl (T), and pyridoxine HCl (P) were prepared in triplicate and placed in 20 mL glass sample vials. Equal parts by mass of all ingredients were used for each mixture. Controlled RH environmental chambers were prepared using saturated salt solutions: potassium carbonate (43% RH), magnesium nitrate (54% RH), sodium bromide (59% RH), cobalt chloride (64% RH), potassium chloride (85% RH), and potassium sulfate (98% RH). The RH of each chamber was verified by digital hygrometer (traceable humidity/temperature/dew point meter, Control Co., Friendswood, TX) or water activity (AquaLab 3TE, Decagon Devices, Inc., Pullman, WA). Powder samples were stored in select RH chambers at room temperature (22 ± 5 °C) for exposure to RHs above, at/near, and below RH_0 or RH_{0mix} for 1, 2, 4, 8, and 12 weeks. Controls were prepared by analyzing powder mixtures immediately after sample preparation. For storage, samples were nitrogen flushed and frozen at −20 °C in 2 mL polypropylene microcentrifuge tubes (DOT

Table 1. RH_0 and a_w Values of Deliquescent Ingredients Measured at 25 °C

ingredient	RH_0 (%)	$a_w \times 100$	lit RH_0 (%) values[a]
thiamin HCl	88	88	89
pyridoxine HCl	–[b]	97	–[c]
sodium ascorbate	86	86	87
fructose	64	63	62

[a] Reported by Salameh et al. (*8*). [b] Above maximum experimental setting of 95% RH. [c] RH_0 for pyridoxine HCl was not previously reported in the literature.

Scientific, Inc., Burton, MI) until analysis. Physical observations such as formation of a solution and mass gain were recorded as indicators of deliquescence and moisture uptake.

pH Determination. An estimation of the pH at the dissolution point of all formulations was obtained by preparing solutions saturated with respect to all ingredients in all formulations and measuring the pH on an Orion model 710A pH/ISE meter (Orion Research, Inc., Beverly, MA) calibrated from pH 2−10.

Initial Moisture Content. The initial moisture of individual vitamins was measured using a microwave moisture/solids analyzer (Smart System 5, CEM Corporation, Matthews, NC) following manufacturer's directions. A sample size of 2−4 g was placed between glass fiber pads and dried at 100% power.

Chemical Stability Determination with HPLC. High-performance liquid chromatography (HPLC) was used to determine vitamins B_1, B_6, and C degradation following the method of Heudi et al. (*12*) with modifications. A chromatographic system including a Waters 510 HPLC pump, a Waters 715 Ultra Wisp sample processor (Milford, MA), and a model 490E programmable multiwavelength detector was used. Separations were achieved using a 3.9 mm × 100 mm (3.5 μm particle size) XTerra RP-C18 column (Waters Corporation, Milford, MA). Resolution of water soluble vitamins was achieved using isocratic elution at 1.0 mL/min with 0.025% trifluoroacetic acid (TFA) (Sigma Co., St. Louis, MO) in double distilled (dd) water (pH 2.6). Detection and identification of the vitamins was performed at 240 nm (for B_1 and C) and 290 nm (for B_6). A 25 μL injection volume was used in this method. Quantification of compounds of interest was performed using multilevel calibration curves constructed with standards of the HCl salt of individual water-soluble vitamins B_1, B_6, and the sodium salt of vitamin C.

Statistical Analysis. A completely randomized three factor factorial design was used for studying the effects of RH, time, and formulation on individual vitamin stability. A MANOVA model was used for this analysis. Individual differences were tested using Tukey's multiple means comparison procedure. All statistical analysis procedures were conducted using PC SAS software and $\alpha = 0.05$.

RESULTS AND DISCUSSION

Deliquescence and Moisture Sorption of Vitamins B_1, B_6, and C. The deliquescence point, RH_0, for each individual ingredient is listed in **Table 1**. Results of a_w measurements for saturated solutions, RH_0 determined from moisture sorption data, and literature values are reported for thiamin HCl, pyridoxine HCl, sodium ascorbate, and fructose. The RH_0 points for these ingredients were 89, 98, 86, and 64% RH, respectively. In general, there is good agreement between experimental and published values, where available (*9*). Additionally, observations of physical stability and changes such as dissolution were recorded for all samples during storage at RHs below, near, and above their deliquescence points. After 12 weeks of storage at 98% RH (above its RH_0 of 89% RH), thiamin HCl formed a clear solution, whereas it remained a powder that appeared to have undergone slight caking following storage at 54% RH (below its RH_0). Sodium ascorbate deliquesced to a dark brown solution, which contained some precipitate during storage at 98% RH (above its RH_0 of 86% RH) and formed a cake at 54% RH. Pyridoxine HCl remained a powder and caked both at and below its RH_0 (at 98 and 54% RH), with some localized liquid droplets visible in samples at the higher RH.

Moisture sorption isotherms for individual ingredients are shown in **Figure 2A**. Fructose had the lowest deliquescence point (at 64%RH), and pyridoxine HCl did not deliquesce within the RH limits of the gravimetric analyzer (up to 94% RH). The initial moisture contents of individual vitamins before drying were 0.43% for thiamin HCl, 0.15% for pyridoxine HCl, and 0.16% for sodium ascorbate (**Table 2**). Total moisture sorption for individual ingredients ranged from 7.0 to 86.4% w/w. Within the experimental parameters, samples arranged from greatest to least moisture sorption (**Figure 2A**) were fructose (86.4% w/w), sodium ascorbate (29.3% w/w), thiamin HCl (18.9% w/w), and pyridoxine HCl (7.0% w/w). This is in accordance with their molecular structures (**Figure 1**): functional groups capable of both accepting and donating hydrogen bonds, ionic charge, and polarity. These observations (**Figure 2A**) are also consistent with the differences in molal solubilities of the ingredients: 22.22 mol/kg for fructose, 3.13 mol/kg for sodium ascorbate, 2.73 mol/kg for thiamin HCl, and 1.08 mol/kg for pyridoxine HCl (*13, 16*).

Deliquescence Lowering in Powder Blends Containing Vitamins B_1, B_6, and/or C. Water activity (a_w) measurements and moisture sorption isotherms were used to estimate RH_{0mix} for binary, ternary, and quaternary ingredient combinations in powder blends. Measured and predicted RH_{0mix} values for deliquescent ingredient powder blends are shown in **Table 3**. This was the first report of the occurrence of deliquescence in powdered mixtures of vitamins C, B_1, and B_6. The a_w values for saturated solutions of mixtures (**Table 3**) are compared to RH_{0mix} values determined by moisture sorption analysis and Ross equation predictions. The Ross equation

$$(a_w)_{mix} = (a_w)_1(a_w)_2(a_w)_i \quad (1)$$

can be used to predict the a_w of a mixture of ingredients from the a_ws of the individual components (*17*). RH and a_w are related as follows: $RH/100 = a_w$. Thus, the Ross equation can also be useful in estimating the critical relative humidity at which deliquescence of a mixture will occur.

The calculated χ is a measure of the deviation of the experimental RH_{0mix} value from the predicted value (*17*), with 1.0 indicating no deviation, and values ranged from 0.96 to 1.28, indicating that there was generally good agreement between predictions and estimated deliquescence points from gravimetric analyses, with greater deviation from the predicted value occurring in ternary or quaternary mixtures (**Table 3**). χ values that deviate significantly from unity indicate the presence of strong solute−solute interactions. The Ross equation does not account for these interactions; thus, differences between predicted and observed RH_{0mix} values may be attributed to this factor (*17*). Deviations may also be due to incomplete contact between all ingredients as the mixtures increase in complexity.

As expected, all ingredient mixtures deliquesced at RHs below the RH_0s of the individual components (**Tables 1** and **3**), and increased moisture uptake was observed as storage RH increased, with minimal moisture uptake below RH_0 or RH_{0mix}, for all samples (**Figure 2** A, **B**, and **C**). Isotherms for thiamin HCl (T) and mixtures TA, TPA, and TPAF are shown in **Figure 2B**. Similar trends were observed for pyridoxine HCl (P) and sodium ascorbate (A) in mixtures not containing T (PA and PAF, data not shown). As the complexity of the mixtures increased (from single ingredient to binary mixtures, ternary, etc.), RH_{0mix} decreased, along with an increase in overall end point moisture uptake. Weight gain during sample storage at

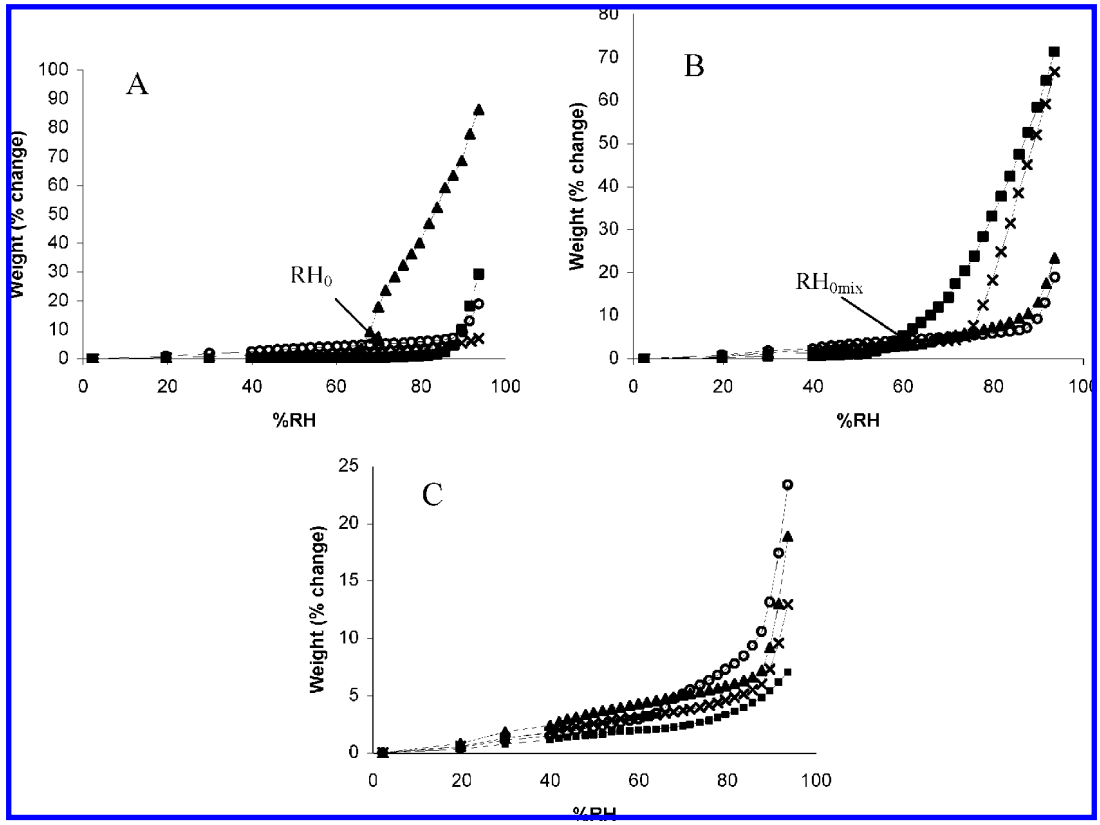

Figure 2. Moisture sorption isotherms of individual ingredients and select blends at 25 °C. Ingredients are abbreviated: T = thiamin HCl; P = pyridoxine HCl; A = sodium ascorbate; F = fructose. (**A**) Moisture sorption isotherms of individual ingredients: thiamin HCl (○), sodium ascorbate (■), fructose (▲), and pyridoxine HCl (×). (**B**) Moisture sorption isotherms of select samples containing thiamin HCl: T (○), TP (▲), TPA (×), and TPAF (■). (**C**) Predicted and observed moisture sorption behavior of a mixture of thiamin HCl and pyridoxine HCl. The predicted values were calculated from the moisture sorption of the individual vitamins. Formulations: TP observed (○), pyridoxine (■), thiamin (▲), and TP predicted (×).

Table 2. Effect of Storage RH and Formulation on the Degradation of Vitamins B_1, B_6, and C Stored at Select RH for 12 Weeks and 22°C[a]

formulation[b]	moisture content (% w/w)	pH	vitamin	storage RH 98	85	64	59	54	43
T	0.43	2.01	thiamin	4.15 ± 4.27 efg	1.18 ± 3.01 fgh			0.00 ± 6.08 i	
P	0.15	2.37	pyridoxine	11.11 ± 2.30 fgh				15.20 ± 1.56 bcdefg	
A	0.16	7.47	ascorbate	97.42 ± 0.01 a	97.41 ± 0.01 a			0.00 ± 5.59 cd	
TF		2.51	thiamin	9.56 ± 4.80 cdefg			0.73 ± 5.10 fgh		0.00 ± 3.29 gh
PF		2.43	pyridoxine	25.28 ± 0.90 a			14.01 ± 0.04 cdefgh		13.75 ± 4.81 defgh
AF		6.68	ascorbate	97.41 ± 0.01 a				0.00 ± 2.56 d	0.00 ± 7.01 cd
TA		5.03	thiamin	29.84 ± 4.35 ab		12.05 ± 0.71 cdefg		0.00 ± 1.25 hi	
			ascorbate	97.42 ± 0.01 a		1.17 ± 1.42 cd		2.67 ± 9.23 cd	
PA		4.58	pyridoxine	15.57 ± 1.27 bcdefg	12.86 ± 1.57 efgh			12.67 ± 2.97 efgh	
			ascorbate	97.42 ± 0.01 a	97.46 ± 0.053 a			0.00 ± 3.88 d	
TP		2.20	thiamin	1.57 ± 5.40 fgh	5.53 ± 1.40 cdefg			0.00 ± 4.95 i	
			pyridoxine	14.53 ± 2.12 bcdefgh	19.35 ± 1.64 abcde			11.94 ± 1.85 efgh	
TPF		2.28	thiamin		6.16 ± 5.73 cdefg			16.82 ± 2.67 bcde	5.28 ± 2.05 efg
			pyridoxine		13.35 ± 1.70 defgh			13.61 ± 0.69 defgh	16.02 ± 2.23 bcdefg
TPA		4.64	thiamin	26.04 ± 1.00 ab		9.61 ± 0.58 defg			4.32 ± 0.42 fgh
			pyridoxine	21.64 ± 1.02 ab		11.29 ± 0.24 fgh			7.03 ± 1.18 h
			ascorbate	97.41 ± 0.01 a		1.08 ± 5.08 cd			0.00 ± 4.74 d
TAF		5.12	thiamin		27.23 ± 2.21 a			14.40 ± 3.63 bcd	9.93 ± 0.70 cdef
			ascorbate		77.62 ± 2.40 b			3.55 ± 4.52 cd	0.00 ± 7.58 cd
PAF		4.60	pyridoxine		20.43 ± 0.81 abcd			14.43 ± 0.22 bcdefgh	15.21 ± 1.98 bcdefg
			ascorbate		77.51 ± 2.11b			0.00 ± 4.23 cd	0.00 ± 6.47 d
TPAF		4.56	thiamin		26.80 ± 1.45 ab		21.30 ± 1.89 abc		6.09 ± 6.35 efg
			pyridoxine		21.35 ± 0.81 abc		18.22 ± 1.71 abcdef		8.56 ± 0.48 gh
			ascorbate		49.84 ± 1.96 b		7.86 ± 2.04 c		0.00 ± 6.90 d

[a] All samples were stored at up to 3 RHs (above, at/near, and below RH_0/RH_{0mix}). Values reported are % degradation from initial amount ± standard deviation. Samples with different letters had significantly different degradation of that vitamin compared to all other formulations containing that vitamin. Initial moisture content reported as % w/w is given for individual vitamins. The pHs of solutions saturated with respect to all ingredients are also reported. [b] Letters listed in formulations indicate the following ingredients: T = thiamin HCl; P = pyridoxine HCl; A = sodium ascorbate; F = fructose.

select RHs, indicative of moisture sorption and expressed as % w/w evaluated over time, demonstrated a general trend of increasing rapidly during the first four weeks before gradually plateauing over the final weeks of storage for many samples

Table 3. RH_{0mix} and a_w Values for Mixtures of Deliquescent Ingredients Measured at 25°C

mixture composition	RH_{0mix} (%) observed	$a_w \times 100$	RH_{0mix} (%) predicted[a]	χ[b]
thiamin HCl/pyridoxine HCl	86	87	87	1.00
thiamin HCl/sodium ascorbate	74	73	76	0.96
thiamin HCl/fructose	59	59	55	1.07
pyridoxine HCl/sodium ascorbate	82	81	83	0.98
pyridoxine HCl/fructose	64	64	61	1.05
sodium ascorbate/fructose	55	55	53	1.04
thiamin HCl/pyridoxine HCl/sodium ascorbate	74	72	74	0.97
thiamin HCl/pyridoxine HCl/fructose	60	65	54	1.20
thiamin HCl/sodium ascorbate/fructose	52	56	47	1.19
pyridoxine HCl/sodium ascorbate/fructose	57	60	52	1.15
thiamin HCl/pyridoxine HCl/sodium ascorbate/fructose	54	59	46	1.28

[a] RH_{0mix} was predicted by the Ross equation (eq 1). [b] $\chi = a_w(\text{observed})/(a^\circ_w)_1(a^\circ_w)_2$.

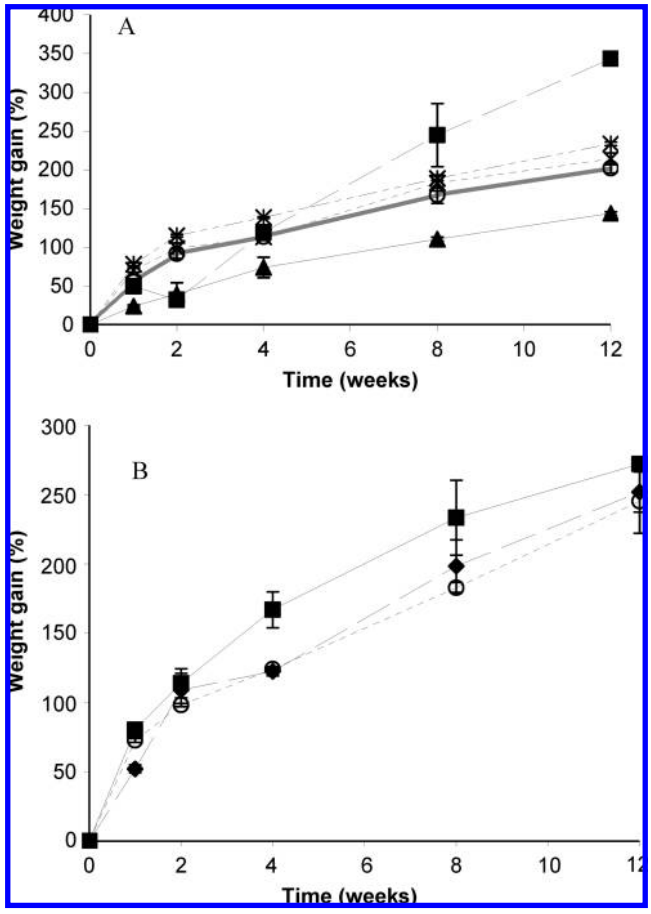

Figure 3. Moisture uptake after 0, 1, 2, 4, 8, and 12 weeks for formulations stored at 98% RH and 22 °C. Ingredients are abbreviated: T = thiamin HCl; P = pyridoxine HCl; A = sodium ascorbate; F = fructose. (**A**)Moisture uptake for thiamin-containing formulations: T (■), TF (×), TP (▲), TA (*), and TPA (○). (**B**) Moisture uptake for ascorbate-containing formulations: A (■), AF (○), PA (◆).

(**Figure 3A** and B). End point weight gain for individual vitamins stored above RH_0 (98% RH) was 343.3% w/w for T, 272.1% w/w for A, and 36.2% w/w for P. Binary mixtures gained 143.7% (formulation TP) to 251.9% w/w (formulation PA). A ternary vitamin blend (TPA) sorbed 202.1% w/w moisture. End point weight gain for thiamin-containing blends above RH_0 or RH_{0mix} ranged from 75.3% to 343.3% w/w (**Figure 3A**). Weight gain for ascorbate blends ranged from 99.3% to 272.1% w/w (**Figure 3B**). Pyridoxine blends demonstrated the least weight gain, ranging from 36.2% to 251.9% w/w. Because of the kinetics of moisture sorption, samples stored over time in controlled RH chambers had greater moisture uptake than observed during dynamic moisture sorption measurements.

Overall, mixtures sorbed more moisture than predicted based on the weight gain of the individual components (**Figure 2C**); that is, moisture sorption in mixtures is synergistic rather than additive. For example, deviations from predicted moisture sorption for formulation TP were observed starting at 70% RH. At this RH, the sample sorbed 1.4% more moisture than predicted. This trend of greater moisture uptake in the experimental sample than predicted continued until the end point (94% RH), at which the sample sorbed 10.5% more moisture than predicted (**Figure 2C**). The deliquescence lowering phenomenon contributes to the enhanced moisture sorption of blends below the RH_0s of the individual ingredients. Vapor sorption in powder blends is known to depend on mixture composition and degree of contact between ingredients (*15*), and the increase in moisture sorption is predicted theoretically by the Gibbs−Duhem equation (*18*). The presence of a second dissolving component can bring more moisture into the system thus permitting further dissolution of the compound of interest. Formation of soluble degradation products can also promote an increase in moisture sorption. Hence, more vitamin enters the solution phase in which it is more susceptible to chemical degradation.

Vitamin Chemical Stability. *Thiamin HCl (Vitamin B_1).* RH and formulation significantly impacted thiamin HCl (T) degradation ($p < 0.0001$). The effects of RH and formulation on T degradation during storage at select RHs for 12 weeks are shown in **Table 2**. Across all treatments, T degradation ranged from 0.0 to 29.8%. T stored above RH_0 (at 98% RH) or near RH_0 (85% RH) exhibited significantly greater degradation compared to samples stored below RH_0 (at 54% RH) ($p = 0.001$ and $p = 0.0209$, respectively). Although T stored at 54% RH caked slightly, no degradation occurred.

Generally, greater degradation occurred in samples stored above RH_{0mix} compared to those stored below, except for formulations TF and TPF. While these formulations sorbed significantly more moisture after 12 weeks of storage above RH_{0mix} compared to storage below RH_{0mix}, thiamin stability was statistically equivalent at all storage RHs for these samples. The stability of some samples exhibiting high amounts of moisture sorption (T, TP, and TF in **Figure 3A**) suggests the occurrence of a dilution effect preventing molecular interactions or formulation effects. Thiamin was least stable in formulations TA and TPA at 98% RH and in TAF and TPAF at 85% RH (**Table 2**). These RHs are above the RH_{0mix} for the given formulations. TA and TPA powder formulations deliquesced and formed very dark brown solutions during the 12 weeks of storage at 98% RH. TAF and TPAF formed dark brown solutions with some precipitate present at 85% RH. Below RH_{0mix}, TA and TPA

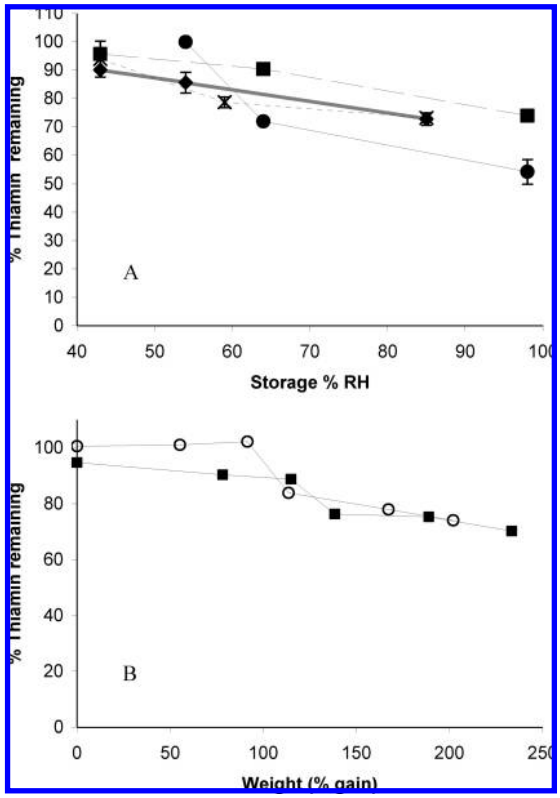

Figure 4. Thiamin stability and moisture uptake profiles in thiamin-containing formulations stored at select RHs for up to 12 weeks at 22 °C. Ingredients are abbreviated: T = thiamin HCl; P = pyridoxine HCl; A = sodium ascorbate; F = fructose. (**A**) Thiamin stability after storage for 12 weeks at select RHs and 22 °C in formulations exhibiting the greatest instability: TA (●), TPA (■), TAF (◆), and TPAF (×). (**B**) Thiamin stability versus weight gain attributable to moisture uptake in select formulations stored at 98% RH and 22 °C for 0, 1, 2, 4, 8, and 12 weeks. Thiamin stability in select formulations: TA (■) and TPA (○).

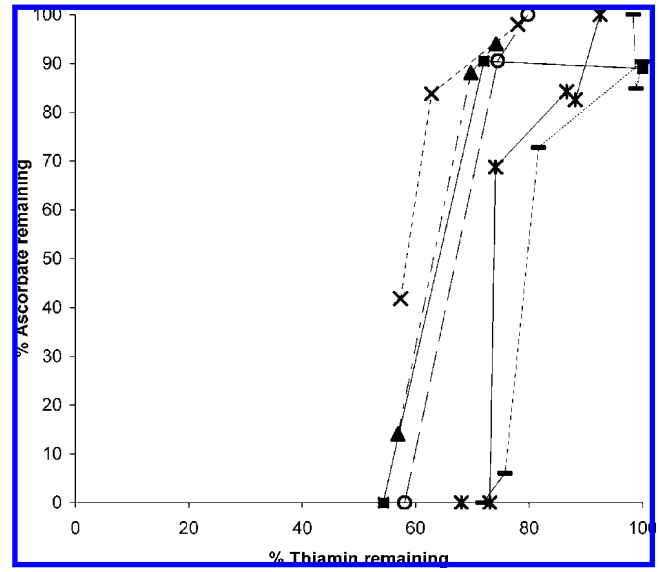

Figure 5. Relationship of % thiamin remaining versus % ascorbate remaining in formulations containing both thiamin and ascorbate: (1) after 12 weeks of storage at 22 °C at select RHs and (2) over time (0−12 weeks) at 98% RH. Ingredients are abbreviated: T = thiamin HCl; P = pyridoxine HCl; A = sodium ascorbate; F = fructose. Select formulations: TA (■), TPA (○), TAF (▲), TPAF (×), 98TA over time (*), and 98TPA over time (−).

remained slightly caked, dry, white powders, while TAF and TPAF formed thick, yellow, pastelike solutions.

Overall, thiamin in mixtures of deliquescent ingredients was most stable when stored below RH_{0mix}, demonstrating the relationship between deliquescence and chemical stability. It is perhaps unsurprising that thiamin HCl is susceptible to degradation above the deliquescence point since a major mechanism of degradation is via hydrolysis. Others have reported thiamin HCl instability in intermediate-moisture foods (*19, 20*). Generally, our findings on thiamin stability in the presence of moisture are in agreement with previous results. Because thiamin HCl degradation was influenced to such a large extent by the deliquescence phenomenon, it is clearly of importance to avoid mixing this vitamin with other ingredients that will substantially decrease RH_{0mix}, unless strict control of environmental RH, well below RH_{0mix}, is possible.

The amount of thiamin remaining in each of the formulations after 12 weeks of storage at select RHs above, near, or below RH_{0mix} is shown in **Figure 4A**. Moisture influenced degradation as indicated by the relationship between weight gain, which is attributable to moisture uptake, and thiamin degradation (**Figure 4B**). Thiamin alone exhibited the greatest moisture uptake after 12 weeks of storage at 98% RH (343.3% w/w) compared to all other thiamin blends (**Figure 3A**). Greater moisture uptake was observed for all samples as RH increased, which correlates with increased thiamin degradation at higher RHs.

End point thiamin degradation in all mixtures and storage RHs was examined to elucidate the impact of individual ingredients on thiamin stability. Formulation appears to affect thiamin stability, evidenced by significant thiamin degradation not attributable to moisture uptake alone. All formulations containing both thiamin and ascorbate demonstrated significantly greater ($p < 0.0001$ to $p = 0.0006$) thiamin degradation compared to formulations without ascorbate present, regardless of moisture uptake. Degradation in these formulations ranged from 26.0 ± 1.00 to 29.8 ± 4.35% compared to 4.2 ± 4.27% for thiamin alone. A plot of percent thiamin HCl remaining versus percent sodium ascorbate remaining indicates an interaction between ascorbate degradation and that of thiamin (**Figure 5**). As the amount of ascorbate remaining decreased, thiamin decreased as well. This indicates a specific role of ascorbate in facilitating thiamin degradation in the solution phase or an alternative mechanism of degradation involving ascorbate.

In the presence of trace metals, ascorbic acid has demonstrated pro-oxidant activity with detrimental effects, oxidizing thiamin to thiochrome in aqueous solutions (*21*). This additional mode of degradation may contribute to observed higher thiamin losses in the presence of ascorbate. Another contributing factor to thiamin stability could be pH. A saturated solution of thiamin HCl had a pH of 2.01, while thiamin formulated with ascorbate ranged in pH from 4.56 to 5.11 (**Table 2**). Thiamin degradation mechanisms are influenced by pH (*5, 23*). At low pH (pH < 5), thiamin tends to degrade via replacement of the primary amino group on the pyrimidine ring by a hydroxyl group, yielding oxythiamin. At intermediate pH (pH 5−8), hydrolysis of the thiazole and pyrimidine moieties at the methylene bridge is the major degradation pathway. The rate of this reaction increases with increasing pH (*5, 19, 22, 23*). Thus, the increase in thiamin degradation extent is consistent with the increase in solution pH caused by the presence of sodium ascorbate. Degradation via this mechanism may also produce deliquescent degradation products, which could further reduce RH_{0mix} and decrease thiamin stability.

Additionally, mixtures containing fructose but not ascorbate (TF, TP, TPF) did not follow the trend of increased thiamin

degradation above RH_{0mix} compared to below RH_{0mix} (**Table 2**). Degradation was not significantly different across storage RH (43−98% RH) for these formulations ($p = 0.9933$ to $p = 1.000$), indicating that fructose may have a slight protective effect on thiamin. Others have reported the potential for fructose addition to prevent thiamin degradation (*5*). Though no mechanism for this effect has been provided, the systems studied in this experiment may attribute the increase in thiamin stability when combined with fructose to an apparent increase in solution viscosity. Rates of thiamin degradation were previously found to decrease in more viscous solutions (*19*). Fructose solutions contained more water above RH_{0mix} and were therefore more dilute with respect to thiamin concentration. Further studies are required to elucidate the role of fructose in the stabilization of thiamin. The addition of pyridoxine HCl did not impact thiamin degradation compared to thiamin stored alone at 98% RH ($p = 1.000$).

Pyridoxine HCl (Vitamin B_6). Pyridoxine end point degradation ranged from 7.0 to 25.3% across all treatments (**Table 2**). In general, pyridoxine was less affected by storage RH than other vitamins. This is probably a consequence of its lower aqueous solubility, higher RH_0, and its major mechanism of degradation being photodegradation. Though degradation did occur, significant differences in percent degradation after 12 weeks above, near, and below RH_0 or RH_{0mix} were only observed for formulations PF, TPA, and TPAF ($p < 0.0001$ to $p = 0.0026$). All other formulations did not demonstrate stability differences in relation to storage RH. These formulations (PF, TPA, TPAF) exhibited the greatest overall pyridoxine degradation (25.3, 21.6, and 21.4%, respectively). Formulation PF exhibited the greatest end point pyridoxine degradation of all formulations (25.3%), though this was not significantly different from degradation in formulations TPA, TPAF, and PAF ($p = 0.7169$ to $p = 1.000$). All other formulations had significantly less pyridoxine degradation ($p < 0.0001$ to $p = 0.0018$).

While mixtures PF, TPA, and TPAF sorbed significantly more moisture than more stable systems, moisture uptake did not correspond to pyridoxine stability across all formulations (**Table 2**). Pyridoxine stability in the presence of moisture is well-documented (*24, 27*. No significant degradation was observed during storage of pyridoxine in aqueous solutions protected from light at 40−60 °C and pH 4−7 (*27*). Gregory and Kirk (*25*) reported that pyridoxine was the most stable form of vitamin B_6 during storage at 37 °C and a_w of 0.6, with no losses observed during the first 58 days after which very slow degradation commenced. Pyridoxine HCl is stable dry and in acidic solutions at room temperature (*26*). In addition, Deritter (*24*) reported that pyridoxine may be classified as stable in pharmaceuticals because it has not demonstrated major degradation issues.

Though some pyridoxine-containing samples with less moisture uptake (P, TPF) also were the most stable, others that sorbed greater amounts of moisture (TP, PA) did not necessarily degrade more (**Table 2**). This suggests the presence of formulation effects. For example, it is interesting to note the relationship between moisture uptake and pyridoxine stability in a binary mixture with ascorbate. Formulation PA had a moisture uptake of 251.9 ± 14.5% w/w after 12 week storage at 98% RH and deliquesced to a dark brown solution. This was significantly different from the moisture uptake (203.3 ± 33.1% w/w) for formulation PF at 98% RH ($p < 0.0001$), but pyridoxine was more stable when combined with ascorbate. Near RH_{0mix}, PA formed a sticky dark brown mass with some liquid present, while below RH_{0mix} it remained a dry, slightly caked, white powder. Pyridoxine degradation did not differ significantly across storage RH for PA formulations ($p = 1.000$) and ranged from 12.7 to 15.6% (**Table 2**). It is hypothesized that ascorbate may be preventing pyridoxine degradation via its antioxidant properties, preventing oxidation of pyridoxine to a less stable B_6 vitamer. Thus, for pyridoxine, addition of ascorbate has a stabilizing effect in contrast to the destabilizing effect observed for thiamin in the presence of ascorbate.

The addition of thiamin or fructose to pyridoxine alone or in mixtures did not impact pyridoxine chemical stability but did alter moisture sorption. The observed degradation in samples P, PA, TP, TPF, and PAF may be attributed to light exposure, as pyridoxine is known to be susceptible to light degradation (*28*). Saidi and Warthesen (*27*) reported pyridoxine losses of 8−22% after a dry model system was exposed to 400 ft-c of light for 15 days.

Sodium Ascorbate (Vitamin C). Sodium ascorbate (A) is sensitive to storage RH both alone and when combined with other deliquescent ingredients (**Table 2**, **Figure 6A**). While ascorbate losses were minimal at storage RHs below the deliquescence point, degradation commenced rapidly once the critical RH was exceeded. The stability of A alone and in mixtures stored at RHs above, at/near, and below the deliquescence point of the individual vitamin and mixtures is shown in **Table 2**. The degradation of A in select mixtures and storage conditions (**Figure 6A**) ranged from 49.8 to 97.4% after 12 weeks of storage above RH_0 or RH_{0mix}. There is a strong relationship between moisture uptake and ascorbate retention in these formulations (**Figure 6B**). Significant A degradation occurred in samples stored above RH_{0mix} compared to samples stored below RH_{0mix} for all ascorbate-containing mixtures ($p < 0.0001$). Additionally, A alone demonstrated significantly more degradation when stored above and near RH_0 versus storage below RH_0 ($p < 0.0001$). Moisture uptake greater than 202.1 ± 6.0% w/w resulted in significantly greater ascorbate losses than in samples sorbing less moisture. Other reports have demonstrated a decrease in ascorbic acid stability with increasing a_w (*6, 29*). Lee and Labuza (*6*) postulated that the increased rate of ascorbic acid loss as moisture content increased was related to a decrease in viscosity and subsequent mobilization of reactants. This could explain some of the ascorbate instability.

Browning was observed in ascorbate and its mixtures when the samples were stored near or above the deliquescence point. In contrast, browning was not observed in ascorbate-containing blends stored below RH_0 or RH_{0mix} (**Figure 6C**). In general, ascorbate alone and in blends formed a brown liquid following 12 weeks of storage at RHs above RH_0 or RH_{0mix}. Kurata and Sakurai (*30*) described one aerobic degradation pathway for ascorbic acid involving oxidation to dehydroascorbic acid (DHAA), followed by hydrolysis to 2,3-diketogulonic acid in the presence of water, then decarboxylation and dehydration to furfural. Furfural may then undergo polymerization or combine with amino acids to produce brown pigments (*31*). According to this mechanism, sufficient water must be present for the hydrolysis step, producing compounds that eventually lead to discoloration. It has been shown that DHAA is very unstable and easily delactonized in aqueous solution (*32*). The instability of ascorbate combined with the observed browning in samples forming a solution above RH_{0mix} suggests that this may be a possible mechanism for ascorbate degradation in these conditions.

The addition of thiamin, pyridoxine, or fructose to form binary mixtures with ascorbate did not affect its degradation ($p = 1.000$). Ascorbate stability did increase in some ternary and quaternary mixtures (PAF, TAF, TPAF); however, this was not attributable to any specific ingredient and appeared to be more

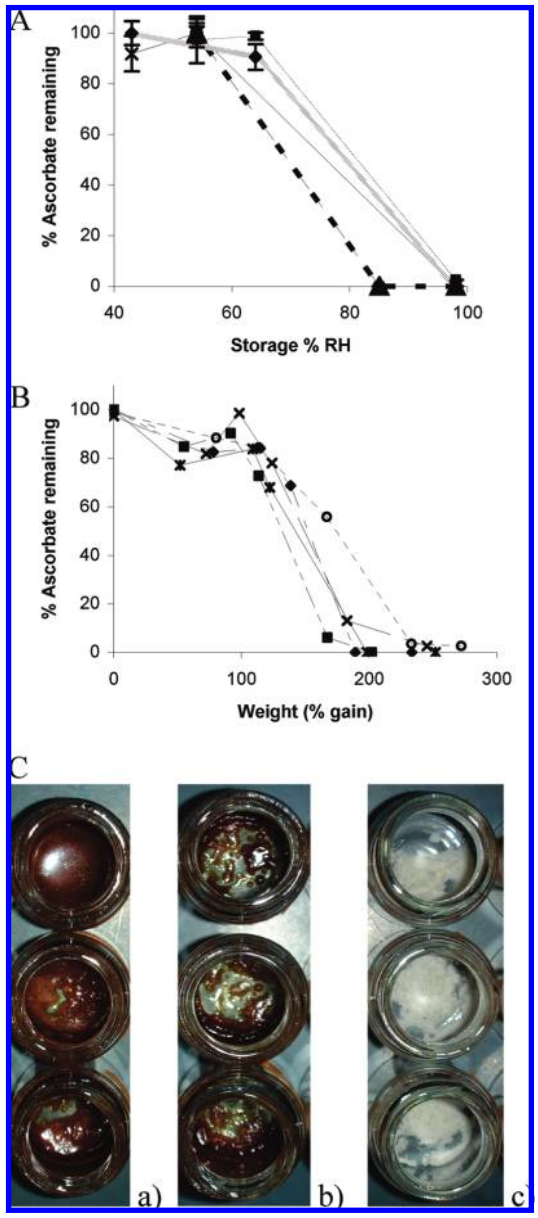

Figure 6. Ascorbate stability and moisture uptake profiles in ascorbate-containing formulations stored at select RHs for up to 12 weeks at 22 °C. Ingredients are abbreviated: T = thiamin HCl; P = pyridoxine HCl; A = sodium ascorbate; F = fructose. (**A**) Fraction of sodium ascorbate remaining after 12 weeks of storage at select RHs (shown on *x*-axis) and 22 °C in select formulations: A (○), AF (×), TA (■), PA (▲), and TPA (◆). (**B**) Ascorbate stability versus weight gain attributable to moisture uptake in select formulations stored at 98% RH and 22 °C for 0, 1, 2, 4, 8, and 12 weeks. Formulations: TA (◆), A (○), AF (×), PA (*), and TPA (■). (**C**) The appearance of sodium ascorbate (in triplicate) following storage at (**a**) 98% RH, (**b**) 85% RH, or (**c**) 54% RH for 12 weeks at 22 °C. Note the dissolution and browning that occurred at 85% and 98% RH.

closely related to the mixture complexity. Additionally, the presence of more ingredients in a formulation may protect ascorbate from oxygen exposure (*33*) thus reducing oxidative degradation and resulting in the higher ascorbate retention observed with some mixtures.

In conclusion, it is apparent that moisture introduced into powder vitamin formulations via deliquescence greatly reduces the stability of thiamin HCl and sodium ascorbate and has less impact on pyridoxine HCl stability. The deliquescence lowering phenomenon contributes to enhanced moisture sorption of vitamin blends at lower RH conditions than for individual ingredients. This will lead to a phase transformation from the more chemically stable solid state to the solution phase during storage at or above the deliquescence point. The microenvironment in the solution phase (pH, viscosity, oxygen content, etc.) will be influenced by the chemistry and concentration of all dissolved solutes, and this can drastically change stability profiles depending on which ingredients are formulated together. Thiamin and ascorbate alone and in mixtures exhibited instability once RH_0 or RH_{0mix} was exceeded, as expected since both are known to be unstable in solution. It is expected that other ingredient forms of these vitamins would follow similar trends. Since an increase in moisture content in powder systems can increase molecular mobility and chemical reactivity, it is also important to consider ingredient interactions. It is a common practice to coformulate these vitamins for multivitamin products or premixes intended for use in food products. The influence of ascorbate on thiamin degradation observed in this study is one such interaction that may be cause for concern when formulating. It is not reasonable to assume these vitamins will remain chemically inert unless RH is maintained below a critical point. To preserve chemical stability and product integrity in vitamin powder formulations, storage and processing conditions should be carefully controlled below the RH_{0mix}.

ABBREVIATIONS USED

T, thiamin HCl; P, pyridoxine HCl; A, sodium ascorbate; F, fructose; TA, thiamin HCl plus sodium ascorbate powder formulations; PA, pyridoxine HCl plus sodium ascorbate powder formulations; TP, thiamin HCl plus pyridoxine HCl powder formulations; TF, thiamin HCl plus fructose powder formulations; PF, pyridoxine HCl plus fructose powder formulations; AF, sodium ascorbate plus fructose powder formulations; TPA, thiamin HCl plus pyridoxine HCl and sodium ascorbate powder formulations; TPF, thiamin HCl plus pyridoxine HCl and fructose powder formulations; TAF, thiamin HCl plus sodium ascorbate and fructose powder formulations; PAF, pyridoxine HCl plus sodium ascorbate and fructose powder formulations; TPAF, thiamin HCl plus pyridoxine HCl, sodium ascorbate, and fructose powder formulations; DHAA, dehydroascorbic acid; RH, relative humidity; RH_0, deliquescence point of an individual crystalline ingredient; RH_{0mix}, deliquescence point of a mixture of crystalline ingredients; B_1, thiamin; B_6, pyridoxine.

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